Lipid packing drives the segregation of transmembrane helices into disordered lipid domains in model membranes

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Conformational switching explains the intrinsic multifunctionality of plant light-harvesting complexes

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The light-harvesting complexes of photosystem I and II (Lhcas and Lhcbs) of plants display a high structural homology and similar pigment content and organization. Yet, the spectroscopic properties of these complexes, and accordingly their functionality, differ substantially. This difference is primarily due to the charge-transfer (CT) character of a chlorophyll dimer in all Lhcas, which mixes with the excitonic states of these complexes, whereas this CT character is generally absent in Lhcbs. By means of single-molecule spectroscopy near room temperature, we demonstrate that the presence or absence of such a CT state in Lhcas and Lhcbs can occasionally be reversed; i.e., these complexes are able to interconvert conformationally to quasi-stable spectral states that resemble the Lhcs of the other photosystem. The high structural similarity of all the Lhca and Lhcb proteins suggests that the stable conformational states that give rise to the mixed CT-excitonic state are similar for all these proteins, and similarly for the conformations that involve no CT state. This indicates that the specific functions related to Lhca and Lhcb complexes are realized by different stable conformations of a single generic protein structure. We propose that this functionality is modulated and controlled by the protein environment.

protein multifunctionality | red forms

Although conformational changes are essential for the function of proteins, little is known about their complex structural dynamics. Protein motions can partially be described by dynamic disorder in the crystalline, glass-like, or liquid states of matter (1–3). A feasible conceptual framework has been developed to visualize these dynamics as transitions between hierarchically ordered minima in the high-dimensional energy landscape of the protein (4, 5). Such a landscape represents all possible energy states as a function of the protein's conformation. The local minima, which signify conformational substates (CSs), are divided by energy barriers into different tiers. The order of a tier is characterized by an average barrier height, a higher barrier of which corresponds to a lower rate of conformational transitions (6). Protein-embedded pigments often serve as effective probes of conformational changes. In particular, strong intrapigment interactions in pigment aggregates can considerably increase the sensitivity of the pigments to the local environment (7). As a result, transitions between CSs are reflected as changes in the pigment electronic states and can be observed as distinct shifts in their absorption and emission energies. In conventional spectroscopy on large ensembles of proteins, energy equilibration after an excitation perturbation is monitored as the average of a very large number of possible trajectories on the energy landscapes of many similar proteins. In contrast, in single-molecule spectroscopy (SMS), the energy landscape of a single protein can be probed. For various pigment-protein complexes, this technique has proven successful to identify conformational changes that occur at rates of 1–10 s^{-1} and which correspond to energy fluctuations of approximately 10–2,500 cm^{-1} (8–14), dynamics that were irresolvable by ensemble techniques.

In this study, similar conformational fluctuations are examined for the photosynthetic pigment-protein complexes that constitute the light-harvesting antennae of plants. These light-harvesting complexes (Lhcs) are associated with two photosystems (PSs), known as PSI and PSII. Upon absorption of a photon, the Lhcs are capable of transferring the excitation energy rapidly and very efficiently to the PSI or PSII core complexes, which are responsible for the primary photochemistry. The outer antenna of PSI comprises the four complexes Lhca1–4, which naturally assemble into the heterodimers Lhca1/4 and Lhca2/3 (15–17). The major outer antenna of PSII, often referred to as LHCII, is a trimer of any combination of the three very similar complexes Lhcb1–3, whereas the minor antenna consists of the three monomers Lhcb4–6, also known as CP29, CP26, and CP24, respectively. The proteins of all these complexes show a high structural homology (18–21) and coordinate the same pigments—namely, chlorophyll (Chl) *a* and *b* molecules and a few carotenoids—in a comparable organization. Despite their structural and compositional similarities, Lhcas exhibit considerably red-shifted and broadened fluorescence emission with respect to Lhcbs. In Lhcas this property originates from a Chl dimer, Chls 603 and 609 (nomenclature of LHCII from ref. 20) (22–24), often called "red Chls", which possesses a charge-transfer (CT) state that strongly mixes with the lower exciton states of the complex (22, 25–28). The resulting low-energy states are often referred to as "red forms." The degree of mixing and accordingly the emission maximum strongly depend on the local protein environment and in particular on the nature of the ligand of Chl 603 (22). Lhca1 and Lhca2, which contain the ligand histidine (H), have low-temperature emission maxima at 690 and 701 nm, respectively, whereas the ligand asparagine (N) in Lhca3 and Lhca4 assists in shifting the maxima to 725 and 732 nm, respectively (29, 30). Changing in Lhca4 the ligand of Chl 603 from an N to an H, thus producing the mutant Lhca4-NH, shifts the low-temperature emission maximum to 686 nm, while an additional emission band is present at approximately 702 nm (22). When the CT character is absent, as in the case of all Lhcbs under normal conditions, the room-temperature emission peaks at approximately 682 nm. However, by applying SMS on LHCII trimers at room temperature, we found that sometimes these complexes switch reversibly to emissive states that are reminiscent of Lhca (11). Moreover, it was suggested that the red emission exhibited by LHCII oligomers can be explained by a similar mixed electronic-CT state (31).

It is well established that Lhcb complexes have a dual function: Apart from efficient light harvesting, they also participate in

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photoprotection by dissipating excess absorbed energy in high-light conditions. In LHCII trimers this is accomplished by conformational switching between a light-harvesting and a dissipating state (32, 33), whereas in the minor antennae formation of a carotenoid radical cation was suggested to be involved (34, 35). Although a photoprotective role has been suggested for Lhca complexes as well (36), little is known about the nature of this process in Lhcas, in particular whether the red Chls play an active role. Participation of the red Chls in singlet quenching has been disputed (37); however, these pigments may be involved in enhanced triplet quenching (38, 39). The general consensus is that the red states serve primarily or solely to significantly enlarge the absorption energy window (40).

This study particularly focuses on the energy fluctuations of the red Chls in dimeric and monomeric units of Lhca and compares the spectral dynamics with those of Lhcbs obtained before (11).

Results

Emission Properties of Single Lhca1/4 Dimers. The fluorescence spectrum of a large ensemble of Lhca1/4 dimers in solution near room temperature is characterized by a broad, structureless, red band at approximately 720 nm, and a considerably narrower blue band at approximately 685 nm. Fig. S1 shows that the room-temperature ensemble-averaged spectra acquired from our SM setup and from a spectrofluorimeter were almost identical, suggesting that the single-molecule environment did not notably affect the average spectroscopic behavior of the complexes as compared to solubilized complexes. However, the spectral shape varied considerably from one complex to the next, in particular the spectral peak position and the relative contribution of the blue and red spectral components. We investigated the time dependence of these spectral properties by resolving the emission spectra on a timescale of 1 s. Large energy fluctuations were observed for the red band, exhibiting peak maxima between approximately 710 and 735 nm, whereas the maximum of the blue band was confined to 685 ± 3 nm (Fig. 1). Nearly 10% of the complexes exhibited large, resolvable spectral shifts of the red band, frequently covering a range of >10 nm (Figs. S2 and S3). These two figures suggest signs of correlated behavior between the peak position and intensity of the red bands. Indeed, considering that the blue and red spectral components originate from different Chl pools in the Lhca1/4 dimer, the intensity of the blue band is expected to scale positively with the energetic position of the red band after complete energy equilibration. Further investigation provided evidence that most complexes displayed such a correlation (Fig. 2*A*), with the exception of <1% of the investigated complexes (Fig. 2*B*).

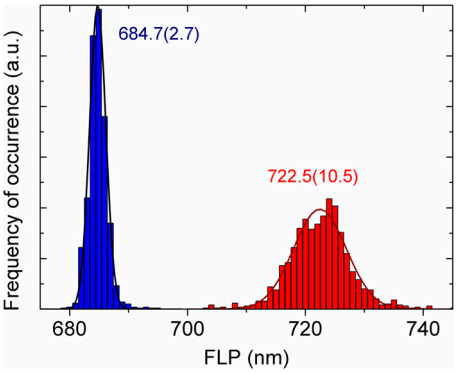

Fig. 1. Distribution of fluorescence peak (FLP) of 66 individually measured Lhca1/4 dimers. An average of approximately 30 spectra was resolved from every complex. The blue and red distributions correspond to the maxima of the blue and red bands of a double skewed Gaussian fit, respectively. Gaussian fits of the distributions are shown; values denote the maxima of these fits, with the FWHM indicated in parentheses.

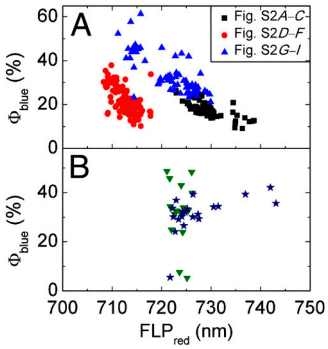

Fig. 2. Relationship between the relative yield of the blue spectral component (Φ_{blue}) and the fluorescence peak of the red spectral component (FLP_{red}) of five single Lhca1/4 complexes. (*A*) Properties of the three complexes in Figs. S2 and S3. (*B*) Two examples of uncorrelated behavior.

Occasionally, a single Lhca1/4 dimer entered an emission state that was almost identical to the typical emission from Lhca1, as demonstrated in Fig. 3. After a few seconds, such a dimeric complex usually switched back to a state with emission properties identical to those of the original state (Fig. 3 and Fig. S4), or sometimes to a different low-energy state (Fig. 3*B*). Although the recovery of the emission was at times delayed by an excursion to a completely quenched state (Fig. 3*A*), the observed switches occurred within a single time step. This rate is noticeably faster than the energy fluctuations displayed in Fig. S2.

Emission Properties of Single Lhca4 Monomers. The low-energy emission of the Lhca1/4 dimer is known to originate from the red Chls of the Lhca4 monomeric subunit (30). The role of Lhca1 in the observed spectral dynamics of Lhca1/4, and whether similar

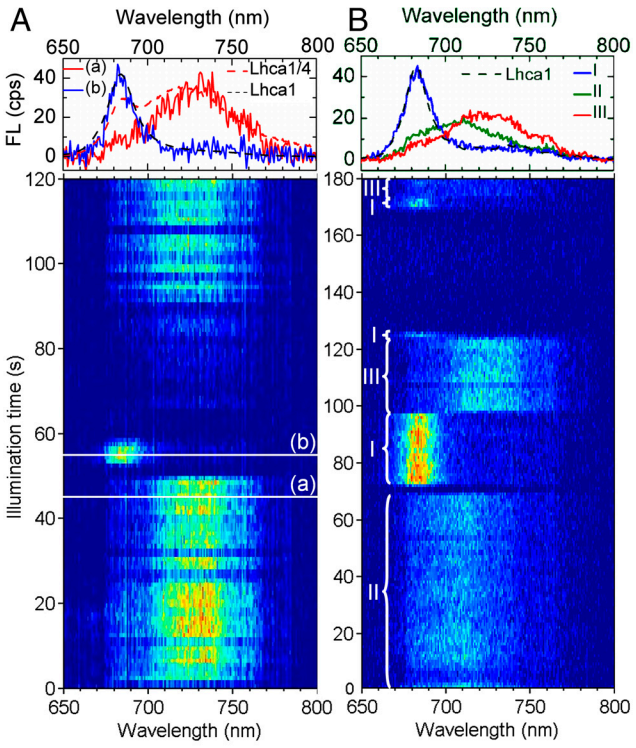

Fig. 3. Representative examples of time-resolved fluorescence spectra from a single Lhca1/4 dimer that switched off its entire red spectral component. Spectra on top represent the spectra at the white, horizontal lines (*A*) or the averages of the fluorescence during the time indicated by I, II, or III (*B*). Black, dashed spectra and red, dashed spectrum (*A*) represent the normalized bulk spectrum at 10 °C of an Lhca1 monomer and an Lhca1/4 dimer, respectively.

dynamics are exhibited when Lhca1 is absent, was of interest. To this end, we investigated single Lhca4 complexes under the same conditions. Fig. 4 indicates that these complexes were also capable of exhibiting significantly varying spectral shapes, each of which was stable for >1 s. The dynamics were such that the fraction of blue emission correlated inversely with the peak position of the red emission (see Fig. S5 for another example of strong correlation between these two spectral properties). Absence of such a correlation was not observed for this complex. Notably, the blue spectral profiles in Fig. 4 *A* and *C* compare well with the blue emission profile of Lhca1/4 in Fig. S2*I*, all of which are characterized by a small red shift and pronounced red tail as compared to an Lhca1 bulk spectrum and a typical LHCII single-molecule spectrum.

Fig. 4 *A* and *C* suggest that Lhca4 is able to perform a transition from a state with emission at 720–730 nm to a state involving an emission band near 705 nm. Although no clearly reversible occurrence of such a transition was observed, a band at around 705 nm is characteristically emitted by Lhca4 complexes and in particular by the Lhca4-NH mutant (25). This finding encouraged further investigation. Fig. 5 shows that not only single Lhca4 and Lhca4-NH complexes were capable of exhibiting a band near 705 nm, but occasionally the broad 720-nm band of a single Lhca1/4 dimer was replaced by a band near 705 nm (Fig. 5*A*). For Lhca4 complexes, the 705-nm emission appeared mostly since the onset of illumination. Remarkably, this band could be switched off and back on by each of the complexes Lhca1/4, Lhca4, and Lhca4-NH under continuous illumination, where the switching occurred within a single time step. For the monomeric complexes, the spectral states lacking the 705-nm band were almost identical to the typical emission spectra of single Lhcb complexes, whereas for Lhca1/4 this state resembled emission from Lhca1.

Lhca4 complexes were significantly less stable than Lhca1/4 under the utilized conditions, surviving typically 2–3 times shorter before irreversible photobleaching and exhibiting a considerable fraction of spectral bluing (see *Materials and Methods*). Less

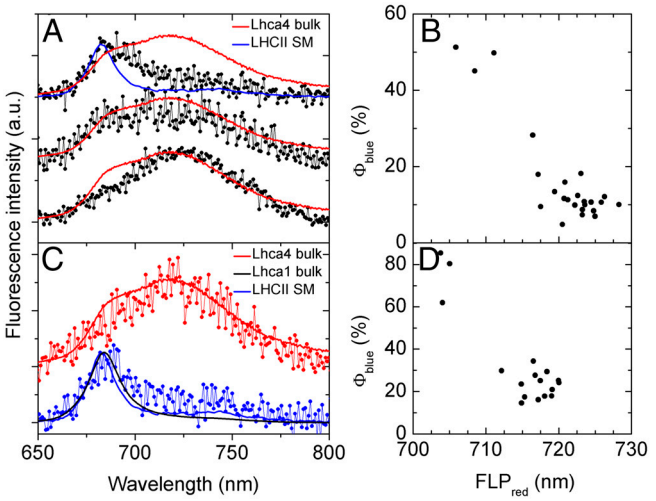

Fig. 4. Time-resolved fluorescence spectra from two individually measured Lhca4 complexes. (*A*) Three different stages of spectral evolution from bottom to top, where averages were taken over spectra with similar profiles. The bottom two spectra were interreversible, and the top spectrum represents the end state before photobleaching. Colored lines denote normalized spectra of bulk Lhca4 in solution at 10 °C (red) and an LHCII trimer measured on the same single-molecule (SM) setup (blue). For clarity, spectra were shifted upward by constant factors. (*C*) Spectral profiles between which a single Lhca4 complex fluctuated reversibly. Thick, blue and red lines are defined as in *A*. Black spectrum represents bulk Lhca1 in solution at 10 °C. (*B* and *D*) Relationship between the relative yield of the blue spectral component (Φ_{blue}) and the fluorescence peak of the red spectral component (FLP_{red}) of the complexes in *A* and *C*, respectively.

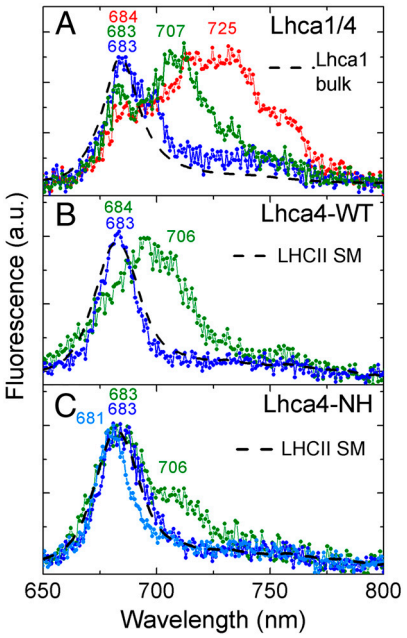

Fig. 5. Different stages of spectral evolution of the emission from three individually measured complexes. Averages were taken over spectra with similar profiles. Maxima of fitted spectral components are indicated on top, with the colors corresponding to the spectra. (*A*) Spectra from a single Lhca1/4 dimer and compared to the normalized spectrum of bulk Lhca1 in solution at 10 °C (black, dashed line). Red spectrum disappeared irreversibly and fluctuations continued between other two spectra. (*B*) Spectra from a single Lhca4 wild-type (WT) complex as compared to a single LHCII trimer under similar conditions (black, dashed line). (*C*) Spectra from a single Lhca4-NH mutated complex as compared to a single LHCII trimer under similar conditions (black, dashed line). Spectrum in light blue originates from a different single complex.

than 10% of the measured Lhca4 complexes exhibited spectra that could be considered useful for our investigation. The fluorescence spectrum of a large ensemble of Lhca4 complexes in solution (Fig. S6) suggests that conformations with blue-shifted emission were favored under the utilized conditions. The Lhca4-NH mutant was even less stable under single-molecule conditions, with only approximately 5% of the investigated complexes exhibiting useful spectral information. Spectral states that resemble a typical Lhcb spectrum were most stable, and spectra that were typically 20% narrower and 2 nm blue-shifted as compared to an Lhcb spectrum were also observed frequently (Fig. 5*C*), though the irreversibility of the latter state points to an unnatural conformation.

Emission Properties of Single Lhca2/3 Dimers. In the utilized single-molecule environment, Lhca2/3 dimers exhibited a similar weak stability as the Lhca4-NH mutant. Nonetheless, the spectral dynamics showed much resemblance with those of Lhca1/4: The amount of blue emission correlated positively with the energy of the red emission band, and instances were observed where the emission band near 720 or 705 nm disappeared reversibly within a single time step (Fig. 6 *A* and *B*). The single, blue-band spectra now corresponded well to the emission from Lhca2 complexes.

Emission Properties of Single Lhcbs. Remarkably, Lhcb complexes have shown the exact opposite switching behavior between single- and double-band spectra as compared to Lhca complexes: Occasionally, a strongly red-shifted emission band appeared temporarily in addition to the characteristic band at 682 nm (11). In addition, similar to Lhca complexes, the spectral profile of the double-band state most frequently remained constant in time, but when it was altered, the energy of the red band correlated

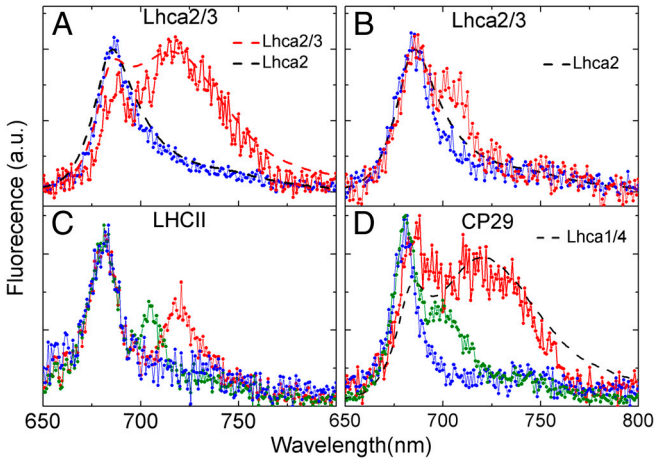

Fig. 6. Representative spectra from individually measured Lhca and Lhcb complexes that switched reversibly between single- and double-band spectral profiles. Averages were taken over spectra with similar profiles. (*A* and *B*) Reversible disappearance of the approximately 720-nm band (*A*) or approximately 705-nm band (*B*) from an Lhca2/3 complex as compared to the bulk spectrum at 10 °C of an Lhca2/3 dimer (red) and an Lhca2 monomer (black). (*C* and *D*) Spectral profiles from a single LHCII trimer (*C*) and two CP29 complexes (*D*) as compared to the bulk spectrum at 10°C of an Lhca1/4 dimer (black). Red and green profiles in *D* originate from different complexes.

well with the intensity of the blue emission band. Fig. 6 *C* and *D* show examples of double- and single-band emission spectra between which single Lhcb complexes switched reversibly. The red bands of Lhcbs were generally narrower than those originating from Lhca1/4 (Fig. 6*C*), but occasionally the widths were comparable (Fig. 6*D*).

Discussion

Spectral Behavior of Single Lhcas. The measured fluorescence spectra are a direct reflection of the energy distribution within the complex at the time of photon emission, averaged over 1 s. Of particular interest are those spectral states that originate from the red Chls—i.e., the excitonic states that are coupled to the CT state of the Chl *a* dimer 603–609 (22, 27). Because of their admixed CT character, these states have acquired the lowest energy (7) and thus contribute to the emission with the highest probability. In the Lhca1/4 dimer, most fluorescence originates from the red Chls of the Lhca4 subunit, and similarly for the red Chls of Lhca3 in the Lhca2/3 dimer.

The spectral behavior of the Lhca1/4 dimer, which was the most stable complex, will be considered first. The behavior exhibited by the Lhca2/3 dimer can be explained in an identical manner. The presented spectral dynamics can be classified into four categories: (*i*) a correlated relationship between the energy variations of the red emission and the relative yield of blue emission, (*ii*) absence of such a correlation, (*iii*) disappearance of the red band within one experimental time step, and (*iv*) an occasional shift of the red band from approximately 720 nm to approximately 705 nm within one time step.

For type *i* behavior, energy variations of the red band between 710 and 740 nm were observed, whereas the spectral fluctuations of the blue band were one order of magnitude smaller. The energy of the red states depends critically on the degree of mixing between the excitonic states and the CT state, which in turn is related to the energetic position of the CT state. Because of the nature of the CT states involving charges, these states interact strongly with their local environment. This strong environmental sensitivity is reflected by the large Huang–Rhys factors that have been estimated for the red emission on the basis of energy selective fluorescence experiments (25, 26, 41). The energy of the CT state is strongly affected by the distance between the red Chls, their relative orientation, and the local charge distribution. As such, subtle changes in the local protein environment can induce sizable shifts in the energy of this state. Indeed, it was shown previously that substitution of Asn, the natural ligand of Chl 603 in Lhca4, with a His leads to the loss of the red-most form because of a small increase in the distance between Chls 603 and 609 (22).

The protein energy landscape model provides an explicable framework to describe the observed energy fluctuations. The considerable amount of energy variations is a clear indication that these systems cannot be considered as rigid entities but that substantial underlying conformational disorder is involved and that the proteins are thus capable of traversing large extents of their energy landscapes. The considerable spectral heterogeneity can be explained by a large number of accessible quasi-stable CSs with different emission properties and differing energies in the energy landscape of the Lhca4 subunit. The large spectral heterogeneity suggests that either the transition frequency between different CSs was often irresolvable on our experimental timescale or that a quasi-continuum of possible energies exists, or a combination thereof. All cases involve relatively low energy barriers between the CSs, reflecting again the sensitivity of the red Chls to their local environment.

It is important to consider the intensity fluctuations of the spectral components in relation to fluorescence intermittency, a property shared by a large range of fluorescing systems and which denotes rapid intensity variations at irregular time intervals (42). Fluorescence intermittency originates from temporal energy dissipation via an energy trap that is intrinsic or coupled to the fluorescing system. Upon excitation of a pigment in Lhca1/4, the energy is equilibrated over the whole complex within approximately 15 ps (43). Because of this energy equilibration, an energy quencher at any site within the complex inevitably leads to energy dissipation. Fluorescence intermittency therefore affects all spectral components equally and is thus distinct from type *i* behavior.

According to the Boltzmann distribution, type *i* behavior is expected for a system in which the excitation energy is fully equilibrated. This is the case for the majority of Lhca1/4 complexes, considering that the main fluorescence lifetime component of approximately 2.5 ns (43) is two orders of magnitude larger than the timescale of energy equilibration [approximately 15 ps (43)]. However, these values represent the averages of a broad distribution, thus allowing the possibility that the energy in a small fraction of complexes may not be equilibrated. Type *ii* behavior is expected only for such a nonequilibrated system and indicates that the rate of excitation energy transfer to the red Chls of Lhca4 is significantly reduced, probably due to some variation in the intermonomeric distance. As expected, type *ii* behavior was not observed for isolated Lhca4 complexes.

Type *iii* behavior, reversible disappearance of the red band, is a particularly interesting property, and most likely involves a change in the nature of the red forms in Lhca4 such that the CT character was completely abolished. Such a conformation would involve that the intermolecular distance of the red Chls is too large and/or their relative orientation is such that no partial CT takes place, a similar situation as for Lhcbs. In addition, the low frequency of this transition and the quasi-stable emission states before and after the transition signify that the corresponding CSs are separated by relatively high energy barriers.

Type *iv* behavior, occasional shifts of the red band from approximately 720 nm to approximately 705 nm, will be considered with the aid of the Lhca monomers. Absence of the 705-nm band in Lhca4 mutants lacking either Chl 603 or Chl 609 (23) strongly suggests that this band originates from the red Chls of monomeric Lhca4 complexes. Indeed, there is a strong indication that all low-energy emission from Lhca complexes originates from the Chl dimer 603–609 (22, 44, 45, 46). The 705-nm species of wild-type Lhca4 and Lhca4-NH can thus be explained in a similar way as the low-emission state of Lhca4 [namely, excitonic-CT

mixing (27)] but corresponding to a different stable conformation that permits a smaller degree of mixing. For the Lhca1/4 dimer, type *iv* behavior can be explained by the Lhca4 subunit adopting a very similar conformation. This behavior could be considered a special case of type *i* behavior. The reversible disappearance of the 705-nm band within one experimental time step can therefore be explained in a similar way as type *iii* behavior. Consequently, the ability of an isolated Lhca4 complex to reversibly switch off its 705-nm emission suggests that it should also be capable of reversibly switching off its emission at lower energies (type *iii* behavior). The natural arrangement of Lhca4 in the Lhca1/4 dimer possibly increases the probability of this behavior.

Conformational Switching Model. All the investigated dimeric and monomeric complexes of the Lhca family have shown the intrinsic ability (or very likely the possibility) to reversibly switch off their red emission. The most plausible explanation is a conformational switch of the Lhca4 complex or subunit between quasi-stable states with and without a CT character, and similarly for the Lhca3 complex or subunit. The reversibility of these switches strongly suggests that these states represent natural states that can be populated in vivo. The nearly identical structures of Lhca1 and Lhca2 suggest an identical behavior of these complexes. This idea is supported by the observation that the Lhca4-NH mutant, which contains the same Chl 603-binding residue as Lhca1 and Lhca2, showed a similar switching on and off of its red emission.

Based on the observed spectral dynamics, we propose in Fig. 7 a simplified protein energy landscape model of all Lhca monomeric complexes. The *x* axis depicts the variable or set of variables that determines the emission wavelength of the complex. Tiers of three orders are shown. The highest tier consists of two CSs, one involving a mixed CT-exciton-state character and one without this

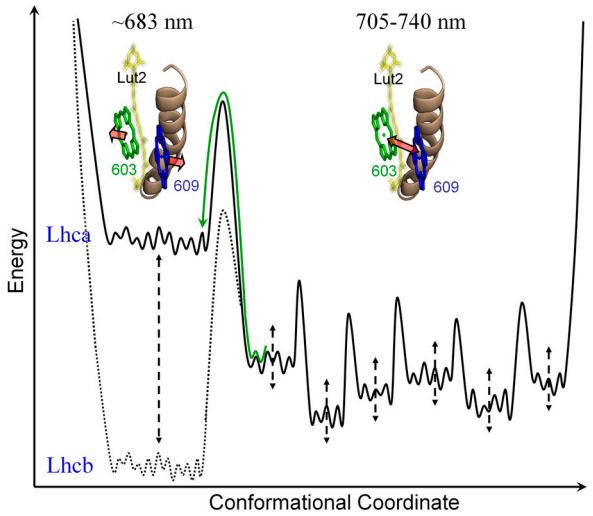

Fig. 7. Cartoon of the energy landscape of a generic protein structure, representing the Lhc proteins of plants. The highest tier contains two quasi-stable conformational states, one of which involves no CT state (*Left*) and the other of which does (*Right*). Tiers of lower order are explained in the text. The energy differences between the landscape of Lhca (solid line) and Lhcb (dotted line) are indicated only on the *Left*. The dashed arrows indicate the energy differences induced by the local protein environment. A structural model (15) of the protein domain in the vicinity of the red Chls of Lhca4 is shown. The position of the carotenoid lutein 2 (Lut2, blurred, yellow) is not resolved in this structure and was taken from ref. 20. The red arrows indicate interactions between red Chls, suggesting a negligible amount of mixing between the CT and exciton states when pointing away from each other. The values of the expected emission maxima associated with the different conformations are shown on top. The green arrow, which partially follows the curvature of the landscape, denotes a possible pathway of a conformational change during which the red emission is switched off. See text for details.

property. These two CSs are separated by the highest depicted energy barrier, which has a very low probability to be crossed. The intermediate tier is shown only for states exhibiting a CT character and comprises six CSs in this cartoon. Transitions between these CSs are observed as spectral jumps of approximately 1–15 nm. The conformation that corresponds to an emission band near 705 nm can be regarded as one of the relatively stable CSs in this tier. The lowest illustrated tier contains the smallest energy fluctuations. Transitions between the CSs in this tier occurred too fast (<1 s) and involved spectral shifts (typically <1 nm) that were too small to be resolved.

The spectral behavior of single Lhcb complexes suggests that the energy landscapes of these complexes are conceptually similar to those of Lhcas, as demonstrated in Fig. 7. First, the spectral signature of Lhca in a blue conformation is almost identical to a typical Lhcb spectrum (Fig. 5). Second, the time-dependent properties of the red emission from Lhcb complexes display much resemblance with the emission from Lhcas. Third, for LHCII it was shown that all emission states above 695 nm very likely involve a CT character and can be explained in an identical manner as for Lhcas (11). Fourth, upon switching into a red conformation, Lhcb complexes often remained in this state for tens of seconds, indicating stable red CSs, similar to the stable blue states of Lhcas. The major difference between the landscapes of Lhca and Lhcb can be regarded as the relative energy difference between the CSs with and without a CT character.

In Fig. 7, the same conformational coordinates have been assumed for all Lhc complexes. Indeed, the property that all these structurally highly homologous complexes are capable of exhibiting very similar spectral dynamics suggests that the corresponding quasi-stable CSs are similar and that their entire energy landscapes are likely very comparable. A single protein structure thus suffices to describe to a high degree all the proteins in the Lhc family. Such a generic protein structure has the intrinsic ability to fluctuate among a large number of possible quasi-stable CSs of its energy landscape. We propose that the subtle structural differences of Lhcb proteins as compared to Lhca proteins are responsible for the former favoring the blue conformation and the latter favoring the red conformations. We also suggest that the equilibrium between the two conformations can be shifted by the local environment of a complex, altering the relative energy of the blue and red conformations, the extent of which is indicated by the vertical, dashed arrows in Fig. 7. In this way, the low-energy emission exhibited by Lhcb complexes under stress conditions and by oligomers of LHCII (47, 48) is probably related to a small shift of the equilibrium to a red CS. Similarly, an Lhca complex can behave like an Lhcb complex when its blue conformation is favored.

Conclusions

We have illustrated that monomeric and dimeric Lhcs of plant PSI exhibit considerable spectral dynamics near room temperature, in particular their low-energy spectral components. PSI and PSII Lhcs can switch reversibly to spectral states that resemble the complexes of the other PS, strongly suggesting fluctuations between conformations with and without red forms. The remarkable similarity in structure and spectroscopic dynamics of these complexes suggests that a single protein structure can represent very well the protein of each of the complexes in the Lhc family. We propose that a subtle perturbation in the physicochemical environment can shift the equilibrium to favor either the blue or the red conformational state, and that this generic protein structure is stabilized in particular conformational states to exhibit the specific functionalities related to Lhca and Lhcb proteins. More generally, the results of this study show that the multifunctionality of a protein can be realized via the control that the environment exhibits over the intrinsic disorder of the protein.

Materials and Methods

Sample Isolation. The Lhca1/4 and Lhca2/3 complexes were purified from *Arabidopsis thaliana* plants as described before (17). The recombinant Lhca4-WT and Lhca4-NH complexes were obtained by overexpression of the *A. thaliana* (WT or NH mutant sequence) apoprotein in *Escherichia coli* and subsequent refolding in vitro with spinach pigments as described in ref. 37. The Lhca4-NH mutant was obtained by site-directed mutagenesis, replacing the Chl-603 binding residue (N47) with a histidine (22).

Experimental Conditions. A home-built confocal microscope was used to observe the fluorescence emission from single complexes. The experimental setup and data acquisition are described in detail in refs. 11 and 49. In short, pulsed excitation light at 630 nm from a $Ti:Al_2O_3$ laser source (Mira 900, Coherent) coupled to an optical parametric oscillator (OPO, Coherent) was used. A near-circular polarization was obtained by using a Berek polarization compensator (5540M, New Focus). Complexes were solubilized in 10 mM Tricine at pH 7.8 and 0.03% n-dodecyl-α-D-maltoside and diluted to a few pM. An immobilization substrate of 3-aminopropyltriethoxisilane (APTES) (Sigma) or poly-L-lysine (PLL) (Sigma) was used. For the Lhca1/4 dimers, excitation intensities of 40–240 W/cm^2 (equivalently 167–1,000 nW) were used. Lhca4 complexes were irradiated with 0.3 μW, and the other complexes were irradiated with 1.0 μW. After immobilization, the sample cell was washed to remove freely diffusing complexes and was deoxygenated by means of the enzymes glucose oxidase and catalase in combination with glucose (all from Sigma). Measurements were performed at 7 °C. Under the utilized conditions, single Lhca1/4 dimeric complexes could be illuminated continuously for tens of seconds and sometimes even a couple of minutes before irreversible photobleaching. Fluorescence emission spectra from large ensembles of complexes were recorded with a Fluorolog 3.22 spectrofluorimeter (Jobin Yvon-Spex).

Data Screening. A single quantum-coupled subunit of PSI was identified by the simultaneous fluctuation of the blue and red spectral bands as the result of fluorescence intermittency. A common irreversible spectral anomaly of complexes in the Lhc family is spectral bluing, characterized by spectra peaking between 650 and 670 nm (11). The fraction of complexes exhibiting spectral bluing was often inversely related to the stability of the complex. Complexes displaying this behavior were disregarded. Spectral profiles consisting of two main spectral components were fitted with a double skewed Gaussian function.

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Supporting Information

Krüger et al. 10.1073/pnas.1105411108

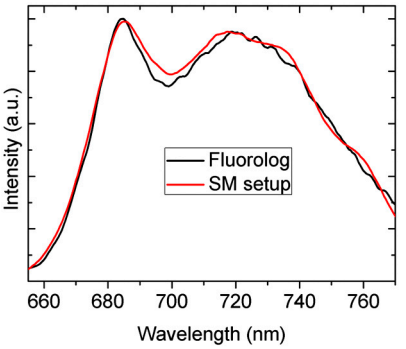

Fig. S1. Fluorescence spectra of ensembles of Lhca1/4 complexes measured in different ways upon 630-nm excitation: A bulk solution measured at room temperature with a spectrofluorimeter (black) and with the single-molecule apparatus (red).

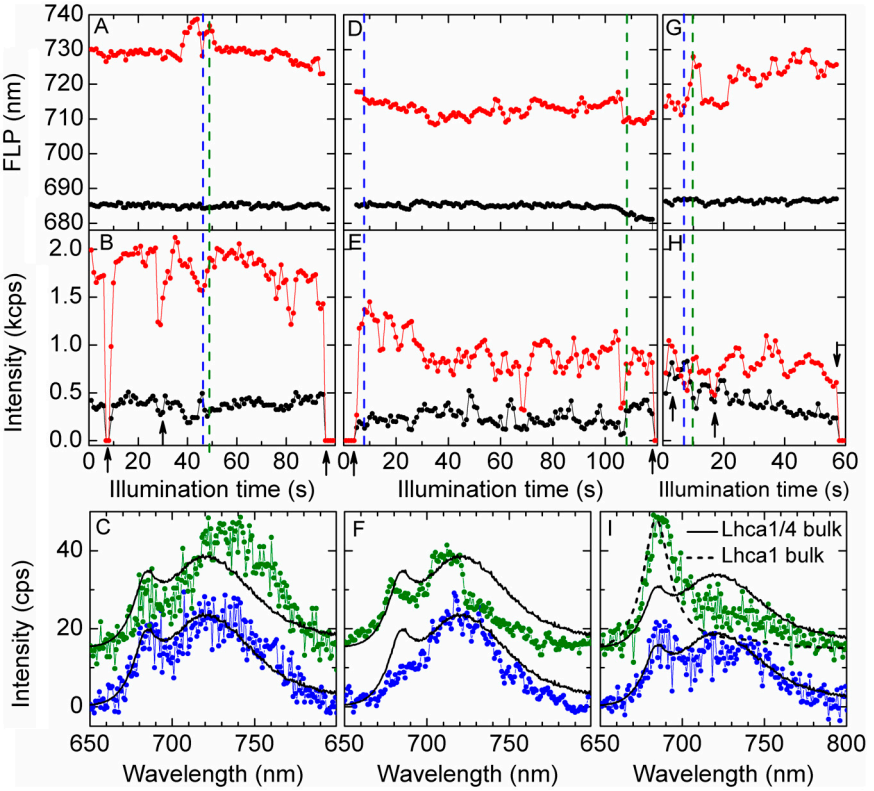

Fig. S2. Three representative examples of time-resolved fluorescence spectra from a single Lhca1/4 dimer under continuous illumination. Each of *A–C*, *D–F*, and *G–I* corresponds to one complex. Fluorescence spectral peak (FLP) (*A*, *D*, and *G*) and intensity (*B*, *E*, and *H*) of the red and blue spectral components (red and black circles, respectively). Intensity is expressed in 1,000 counts per second (kcps) and is integrated over all wavelengths. Correlated fluctuations in the intensity of the blue and red spectral components are indicated by black arrows. The presence of such correlations signifies that the emission originated from a single coupled system. Apart from this behavior, the wavelength of the red spectral component often correlates with its intensity and anticorrelates with the intensity of the blue spectral component. (*C*, *F*, and *I*) 1-s resolved fluorescence spectra that correspond to the dashed lines in the upper panels with the corresponding color. Black spectra represent a normalized bulk spectrum of Lhca1/4 at 10 °C, or of Lhca1 in *I* (dashed line). Green spectra and corresponding bulk spectra are shifted by 15 cps for clarity.

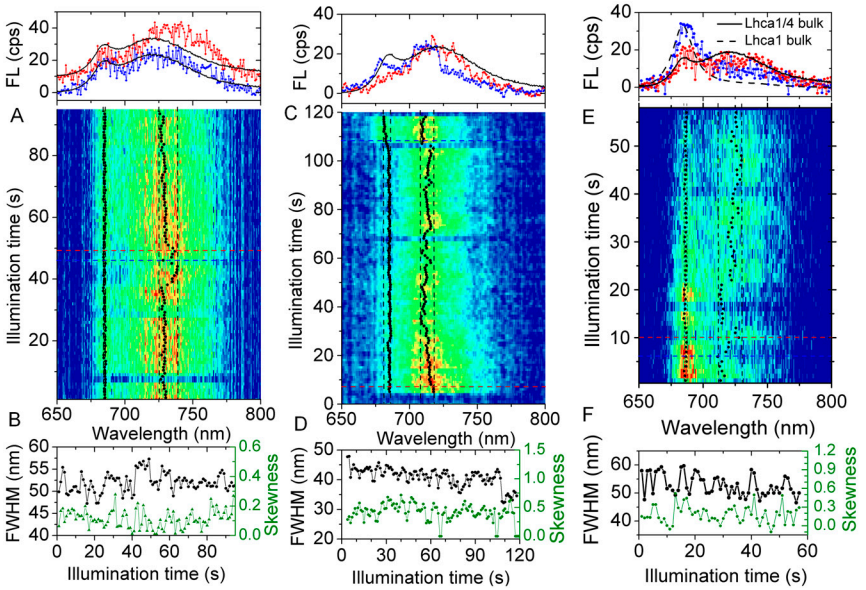

Fig. S3. Time-dependent alterations of the fluorescence spectral profiles of the same complexes used in Fig. S2, indicating strong fluctuations of the spectral shapes. *A* and B, *C* and D, and *E* and *F* correspond to Fig. S2 *A–C*, *D–F*, and *G–I*, respectively. (*A*, *C*, and *E*) Spectral time traces, with the peak positions denoted by black circles and the extents of the peak variations by the black dashed lines. Spectra on top represent the fluorescence intensity (FL) in counts per second (cps) at the horizontal dashed line with the corresponding color. The black spectra represent a normalized bulk spectrum of Lhca1/4 at 10 °C, or a bulk spectrum of Lhca1 (dashed line in *E*). Red spectrum in *A* is shifted by 10 cps for clarity. (*B*, *D*, and *F*) Full width at half maximum (FWHM) (black) and skewness (a measure of the asymmetry of the spectrum) (green) of the red spectral bands in *A*, *C*, and *E*. The FWHM of the red band is often correlated with its emission peak (Fig. S2 *A*, *D*, and *G*), whereas the largely fluctuating skewness exhibits a more complex behavior.

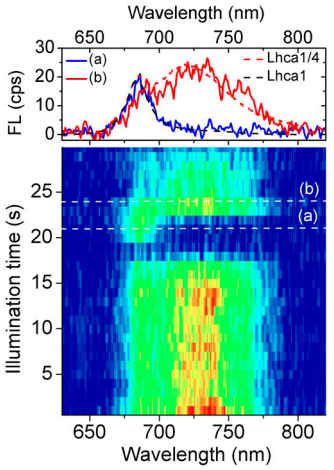

Fig. S4. Representative example of time-resolved fluorescence spectra from a single Lhca1/4 dimer that switched off its entire red spectral component. Spectra on top represent the spectra at the white, horizontal, dashed lines. Black, dashed spectrum and red, dashed spectrum represent a bulk spectrum at 10 °C of an Lhca1 monomer and an Lhca1/4 dimer, respectively.

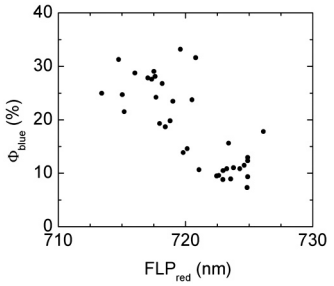

Fig. S5. Relationship between the relative yield of the blue spectral component (Φ_{blue}) and the fluorescence peak of the red spectral component (FLP_{red}) of a single wild-type Lhca4 complex.

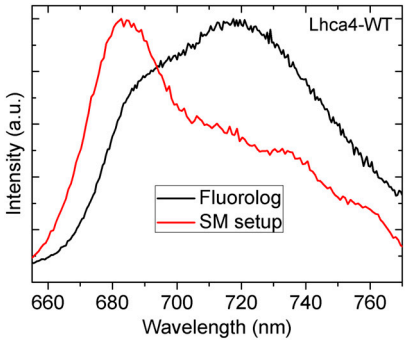

Fig. S6. Fluorescence spectra of large ensembles of Lhca4 wild-type (WT) complexes measured in different ways upon 630-nm excitation: A bulk solution measured at 10 °C with a spectrofluorimeter (black), and with the single-molecule apparatus (red).